

Development, Survival and Fitness Performance of *Helicoverpa zea* (Lepidoptera: Noctuidae) in MON810 Bt Field Corn

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ABSTRACT *Helicoverpa zea* (Boddie) development, survival, and feeding injury in MON810 transgenic ears of field corn (*Zea mays* L.) expressing *Bacillus thuringiensis* variety *kurstaki* (Bt) Cry1Ab endotoxins were compared with non-Bt ears at four geographic locations over two growing seasons. Expression of Cry1Ab endotoxin resulted in overall reductions in the percentage of damaged ears by 33% and in the amount of kernels consumed by 60%. Bt-induced effects varied significantly among locations, partly because of the overall level and timing of *H. zea* infestations, condition of silk tissue at the time of egg hatch, and the possible effects of plant stress. Larvae feeding on Bt ears produced scattered, discontinuous patches of partially consumed kernels, which were arranged more linearly than the compact feeding patterns in non-Bt ears. The feeding patterns suggest that larvae in Bt ears are moving about sampling kernels more frequently than larvae in non-Bt ears. Because not all kernels express the same level of endotoxin, the spatial heterogeneity of toxin distribution within Bt ears may provide an opportunity for development of behavioral responses in *H. zea* to avoid toxin. MON810 corn suppressed the establishment and development of *H. zea* to late instars by at least 75%. This level of control is considered a moderate dose, which may increase the risk of resistance development in areas where MON810 corn is widely adopted and *H. zea* overwinters successfully. Sublethal effects of MON810 corn resulted in prolonged larval and prepupal development, smaller pupae, and reduced fecundity of *H. zea*. The moderate dose effects and the spatial heterogeneity of toxin distribution among kernels could increase the additive genetic variance for both physiological and behavioral resistance in *H. zea* populations. Implications of localized population suppression are discussed.

KEY WORDS *Helicoverpa zea*, plant resistance, transgenic corn, moderate dose

GENETICALLY ENGINEERED CORN plants (*Zea mays* L.), produced from the gene transformation event MON810 (Monsanto Company, St. Louis, MO), expressing *Bacillus thuringiensis* variety *kurstaki* Cry1Ab endotoxins, provide high dose and season-long control of European corn borer, *Ostrinia nubilalis* Hübner (Koziel et al. 1993, Armstrong et al. 1995). High dose has been defined as 25 times the concentration required to kill 99% of the most vulnerable stage of susceptible individuals (Gould 1994, SAP 1998). MON810 corn expresses the protein toxin in the silk tissue and kernels as well as leaves, tassels, and stalk, thus it is also biologically active against the corn earworm, *Helicoverpa zea* (Boddie) (Sims et al. 1996). However, many *H. zea* larvae are able to survive and complete development in Bt corn ears (Sims et al. 1996, Storer et al. 2001) but may suffer negative fitness effects because of sublethal exposure.

Previous studies have shown that *H. zea* individuals surviving sublethal exposure to Bt toxins exhibit fitness disadvantages including prolonged development, decreased larval weight, and reduced fecundity (Hornby and Gardner 1987, MacIntosh et al. 1990, Halcomb et al. 1996, Sims et al. 1996, Williams et al. 1998), and increased exposure to natural enemies (Mascarenhas and Luttrell 1997). Sublethal effects may have a greater effect on survival and reproduction of susceptible *H. zea* individuals compared with resistant individuals, thus increasing the selective fitness differential between these genotypes. According to Sims et al. (1996), resistance management studies on transgenic Bt corn should focus on the proportion of *H. zea* larvae completing development and how well they pass resistant genes to the next generation. This information can provide valuable insights in assessing resistance risks, predicting localized population suppression, and defining appropriate resistance management tactics.

Reported here are studies of the lethal and sublethal effects of MON810 Bt corn on *H. zea*. Specifically, various measures of *H. zea* larval development, survival, and feeding injury in Bt and non-Bt ears were compared at several geographic locations over two growing seasons. Also, the effects of Bt exposure on postlarval development and survival, pupal weight,

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adult fecundity and egg viability, and performance of F_1 larvae were examined to test the hypothesis that sublethally exposed larvae exhibit fitness advantages in later life stages.

Materials and Methods

Plot Design. Four randomized blocks of two corn types (Bt and non-Bt) were planted at four locations (Upper Marlboro and Beltsville, MD, Suffolk, VA and Corapeake, NC) in 1996 and 1997. In both years, late plantings were made in mid-May to early June, so that silking would coincide with the summer moth flight in late July. In 1996, plots within each block consisted of the inbred MON810 event 'Ezra' and its non-Bt isolate. Each plot was eight rows by 15–23-m long and spaced 75 cm apart. Plots were separated by a 5-m buffer strip of Sudex grass *Sorghum bicolor* (L.) Moench to reduce cross-pollination. The same locations and experimental design were used in 1997, except the North Carolina site was not included and plots consisted of 12 rows each of the YieldGard 'Pioneer Brand 33V08' (Bt) and 'Pioneer Brand 3394' (non-Bt) hybrids. Plots in 1997 were 30 m long with a 6-row buffer of sterile corn ('Funks 4394') planted between each plot. In all plantings, seeds were treated with standard fungicidal protectants and planted at a rate to achieve 65,000 plants per hectare. The fertility and herbicide regimes were applied according to agronomic practices recommended for the field conditions at each location.

Ear Sampling. In both years, destructive sampling was conducted twice weekly beginning one week after 80% silking by randomly removing 25 ears per plot. The silk tissue of each ear was carefully examined for *H. zea* larvae and injury before the husk was split open to expose the silk tube and kernels. Data were recorded on the number of each instar, and the location and amount of kernel injury. Injury was visually estimated to the nearest 0.5 cm² of kernel surface area consumed. The location of injury was recorded as: 1) ear tip, including the small, underdeveloped kernels at approximately the top 3 cm of the ear, 2) upper ear, consisting of the top one-half of the fully developed kernels and, 3) the lower one-half of the ear. Ear sampling was conducted over a four and 8-wk period in 1996 and 1997, respectively, until *H. zea* larvae were no longer present in the plots.

Feeding Patterns. In 1996, data were collected at the Maryland locations to characterize the spatial pattern of feeding injury on ears. Line drawings of the kernel injury caused by individual larvae were hand-drawn to scale on cm² graph paper. Each drawing was scanned by a video digitizing system and analyzed by SigmaScan Pro 2.0 (Jandel Scientific 1987). The following parameters were computed: 1) area; 2) perimeter or total length of the outer boundaries; 3) compactness, expressed on a numeric index tending toward infinity as the pattern became more scattered; 4) shape, as an index ranging from 0 (completely linear as a line) to one (perfect circle); 5) major axis length; and 6) minor axis length. Only drawings of feeding damage caused by one larva of a known instar were analyzed.

Larval Development. Age-specific densities of *H. zea* larvae developing on Bt and non-Bt ears were computed by the 'area under the curve' method (Southwood 1966, Pedigo and Zeiss 1996). To simplify data compilation and analysis, age specific groups of early (first-third) and late (fourth-sixth) instars were pooled within each sampling time and plotted over a physiological scale of cumulative degree-days for each replicate plot. Daily maximum and minimum temperatures recorded at each location were used to calculate degree-days using a single sine method (Baskerville and Emin 1969) with a lower developmental threshold of 12.6°C and an upper threshold of 33.3°C (Hartstack et al. 1976). For each replicate, the area under the population density curve for each age group was computed (Enstat 4.0, Kluberantz and Evanson 1996) and divided by the number of degree-days required for larvae to pass through that age group. For *H. zea* larvae feeding on non-Bt corn, the values of 81.7 and 120.6 were used as the degree-day requirements for early and late larvae, respectively (Hartstack et al. 1976). Because larval development in Bt corn is slower (Sims et al. 1996, Storer et al. 2001), degree-day requirements were estimated by rearing *H. zea* larvae on a meridic diet (Southland, Inc., Lake Village, AK) incorporated with dried Bt or non-Bt leaf tissue. Leaf powder was prepared by growing Bt, Pioneer 33V08, and non-Bt, Pioneer 3394, plants in the greenhouse until the V4 stage. Plants were cut at 3 cm above the soil surface, placed in paper bags, and dried in a forced air oven at 55°C for 48 h. The dried tissue was then processed through a tissue grinder using a 1 mm sieve plate and kept in a freezer at -80°C until used. Range-finding bioassays were conducted with 5, 15, 20, 30, and 45 g of dried Bt and non-Bt leaf powder per liter of diet to measure *H. zea* development and survival. Feeding on diet incorporated with 20 g of Bt powder resulted in 70–80% larval mortality and 5–7 d developmental delays, which were responses observed in Bt ears in this study and similar to those reported by other studies (Sims et al. 1996, Storer et al. 2001). Using this concentration, 60 *H. zea* neonates were individually reared in 36-ml Solo cups containing either Bt or non-Bt diet in an incubator at 25°C, 75% RH, and a photoperiod of 16:8 (L:D) h. Larvae were checked daily for stadiar changes until the last instar completed development. Relative differences in the average developmental times for early (1–3) and late (4–6) instars on both diets were used to proportionately adjust the degree-day requirements for early and late larvae feeding on Bt corn.

Fitness Measurements. To explore possible fitness effects because of sublethal exposure, late sixth instars approaching the prepupal stage were collected from Bt and non-Bt plots at the Maryland locations. Larvae were placed in 36-ml Solo cups containing a meridic diet (Southland Inc., Lake Village, AK), coded according to location and treatment plot, and then brought to the laboratory. Cups were held in an incubator (25°C, 70% RH, and a photoperiod of 16:8 (L:D) h and examined daily to determine when pupation or death occurred. Records were kept on the

days to pupation, sex of pupae based on morphological features described by Ditman and Cory (1931), and pupal weight. Pupae were surface sterilized using a 0.2% bleach solution for 3 min to reduce disease infections, rinsed in tap water for 5 min, and placed into ventilated 120-ml Solo plastic cups in the same incubator. Pupae were checked daily for emergence or death.

At emergence, cohorts of 5 male:5 female moths were marked on their wings with a Sharpie permanent marker (Sanford Corp., Bellwood, IL) to track the number of dying moths in time. All moths used in each cohort were virgin adults of the same age (± 1 d) and collected from the same location and treatment plot. Cohorts were placed in oviposition chambers consisting of white plastic buckets (17 cm \times 15 cm dia) with 5-cm dia holes covered with saran screening around the sides. The center was cut out of the bucket lids so that only the rim remained. A sheet of white dacron chiffon was placed loosely over the top of the bucket and secured by the lid rim to provide an oviposition surface. Strips of chiffon also hung down inside the bucket to provide additional ovipositional sites. Chambers were kept in an incubator held at 25°C, 70% RH, and a photoperiod of 16:8 (L:D) h. Moths were fed a sugar water solution on a cotton ball as a source of nutrition. Twelve mating cohorts each of Bt and non-Bt moths in 1996 and 7 in 1997 were examined daily to record fecundity and survival over time. Eggs were collected and counted daily from the chiffon that was replaced with fresh pieces. Daily oviposition per female was adjusted for the number of marked females that died during each collection interval. Eggs were placed in 18 \times 15 \times 5 cm clear plastic containers with small holes in the lid, and reared in an incubator kept at 25°C, 70% RH, and a photoperiod of 16:8 (L:D) h. The cumulative number of larvae hatching was tallied after two and 3 d in 1996 and after 2, 3, and 4 d in 1997 to estimate percent eclosion.

F₁ Performance. Laboratory tests were conducted in 1996 to determine if sublethal exposure to Bt corn affects development and performance of the F₁ generation of *H. zea* larvae. Soybean [*Glycine max* (L.) Merr.] was selected as the food source because late summer larvae normally infest this crop in the mid-Atlantic region. Bioassay feeding tests arranged in a completely randomized design were repeated five times during August and September. In each test, 25 neonate larvae which were the F₁ progeny of moths reared from larvae surviving either Bt and non-Bt corn were allowed to feed for 9 d on soybean leaves. Tests were conducted in 120-ml Solo plastic cups with moist filter paper and provisioned with two leaflets of V5-stage soybean (Lambert et al. 1992). Fully expanded upper leaflets were excised under water from plants collected in the field, thoroughly rinsed, and placed on paper towels to dry. Two neonates were placed in each cup directly onto the excised leaves, but after 3 d, one larva was randomly removed to avoid cannibalism. Leaves were replenished daily. After 9 d, weight gain was recorded as a fitness measure of performance of the F₁ larvae.

Data Analysis. The data collected each year on *H. zea* development and ear damage were analyzed separately for each location, whereas the fitness data collected in the laboratory were pooled over years. Cumulative measures of the percentage of damaged ears and amount of kernel injury were analyzed only for data recorded at the final sampling date. Differences in instar, number of larvae per ear, and percentage of ears infested were compared across all sampling dates. The location of kernel injury was compared between treatments for two sampling dates, coinciding with the peak of fifth instars in Bt and non-Bt corn at each location.

All data were tested for normality and homogeneity of variances using Spearman's Rank Correlation and Shapiro-Wilk's W test. For data not meeting the assumptions of analysis of variance (ANOVA), variances were grouped before analysis (Russek-Cohen and Douglas 1999). The PROC MIXED procedure of ANOVA (SAS Institute 1995) was used to test for differences in laboratory and field parameters between Bt and non-Bt treatments. Means were separated following a significant *F* test by using the method of LSDs ($P \leq 0.05$). In addition, the repeated measures option using the first order autoregressive covariance structure was used to compare instar, number of larvae per ear, and fecundity of moths over time. The compound symmetry covariance structure was used to compare the number of Bt and non-Bt ears infested with larvae across the growing season. Number of ears infested and instars were statistically analyzed for the Maryland locations only. Comparisons of sex ratio and mortality of immature stages in the laboratory were analyzed using chi-square test (SAS Institute 1995). For analysis of parameters characterizing feeding patterns, each instar (second through sixth) was analyzed separately by the Student's *t*-test (SAS Institute 1995) to indicate potential differences in kernel feeding behavior between larvae on Bt and non-Bt corn.

Results

Ear Damage. In 1996, Bt expression in MON810 corn generally reduced the percentage of ears damaged by *H. zea* (Fig. 1), but differences were dependent on location as indicated by the significant interaction ($F = 3.9$; $df = 3, 12$; $P = 0.037$). In 1997, only main effects for corn type ($F = 35.3$; $df = 1, 18$; $P = 0.001$) and location ($F = 10.4$; $df = 2, 18$; $P = 0.001$) were significant. Overall 33% fewer ears were damaged in the Bt plots, and significantly more ears of both hybrids were damaged at the Upper Marlboro and Suffolk locations (35 and 39%, respectively) compared with the Beltsville location (27%).

The surface area of kernels consumed by *H. zea* was also significantly reduced by MON810 Bt corn at most locations (Fig. 2). In 1996, the corn type \times location interaction was significant ($F = 33.1$; $df = 1, 780$; $P = 0.001$) indicating that area consumed was significantly reduced in Bt corn at all locations except at Corapeake, NC. In 1997, relative reductions in the amount of kernel feeding in Bt corn were similar across loca-

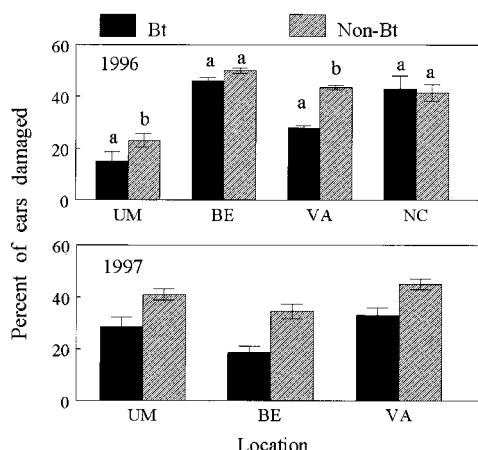


Fig. 1. Mean percentage (\pm SEM) of MON810 Bt and non-Bt ears damaged by *H. zea* in 1996 and 1997 at the Upper Marlboro (UM), Beltsville (BE), VA (VA), NC (NC) locations. Mean bars within each year with no letter or the same letter are not significantly different ($P < 0.05$).

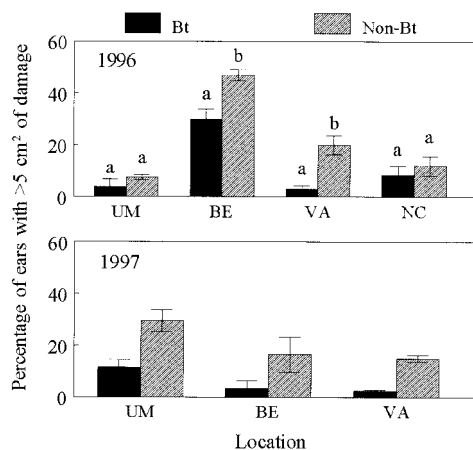


Fig. 3. Mean percentage of ears (\pm SEM) with >5 cm² kernel in MON810 Bt and non-Bt ears damaged by *H. zea* in 1996 and 1997 at the Upper Marlboro (UM), Beltsville (BE), VA (VA), NC (NC) locations. Mean bars within each year with no letter or the same letter are not significantly different ($P < 0.05$).

tions. The surface area of kernels consumed averaged 1.5 cm² in Bt ears and 3.8 cm² in non-Bt ears ($F = 72.3$; $df = 1, 585$; $P = 0.001$). Differences in kernel injury were also significant among locations ($F = 72.3$; $df = 2, 9$; $P = 0.007$).

The number of ears with >5 cm² of kernel damage was tallied and expressed as the percentage of ears within which *H. zea* reached the late instar stage. Generally, if a larva survived to the sixth instar, it consumed >5 cm² of surface area and usually developed to prepupa and exited from the ear. Many ears counted either contained a sixth instar or exhibited extensive kernel damage associated with an exit hole. In 1996, a significant corn type \times hybrid interaction

($F = 4.7$; $df = 3, 12$; $P = 0.022$) indicated that differences between corn types were not the same among locations, but the overall trend was for a significantly lower percentage of Bt ears with >5 cm² of damage (Fig. 3). In 1997, only the main effects for corn type ($F = 23.0$; $df = 1, 18$; $P = 0.001$) and location ($F = 6.1$; $df = 2, 18$; $P = 0.010$) were significant (Fig. 3). The incidence of major ear damage was 70% less in Bt ears (6%) than in non-Bt ears (20%), and the Upper Marlboro location had significantly more ears with major kernel damage (21%) than the VA and Beltsville locations (9 and 10%, respectively).

The distribution and pattern of ear damage by *H. zea* was significantly altered by Bt expression in the kernels. Although data were not statistically analyzed, 43% of the non-Bt ears were damaged by *H. zea* larvae on the upper ear below the tip, while larvae penetrated only 28% of Bt ears below the ear tip. Differences in measurements of feeding patterns between corn types are summarized in Table 1. Except for second instars, the spatial patterns of kernels damaged by each instar in Bt corn were significantly less compact, more linear, and had a greater perimeter length.

Larval Development. Bt expression had a significant impact on the number and stadial development of *H. zea* larvae feeding in corn ears. In 1996, there was a significant location \times time \times corn type interaction for percentage of ears infested with one or more *H. zea* larva ($F = 4.8$; $df = 3, 83.9$; $P = 0.004$) (Fig. 4). The same interaction effect was significant for number of larvae per ear ($F = 8.9$; $df = 3, 46.6$; $P = 0.001$) (Fig. 5). Infestations in non-Bt corn were generally higher than infestations in Bt corn earlier in the season. However, the number of larvae found in Bt ears exceeded levels in non-Bt ears toward the end of the season. Most larvae completed development and exited from non-Bt ears within several weeks after silking, while the slower developing larvae were present in Bt ears

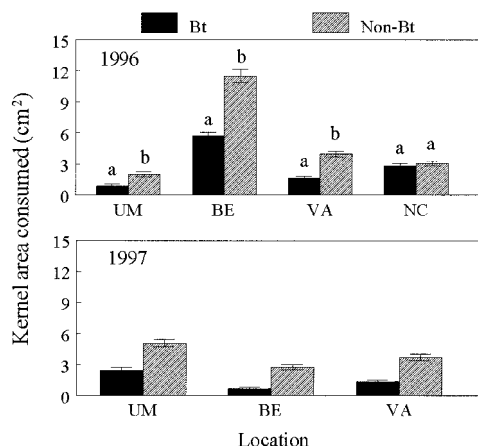


Fig. 2. Mean surface area of kernels consumed (\pm SEM) in MON810 Bt and non-Bt ears damaged by *H. zea* in 1996 and 1997 at the Upper Marlboro (UM), Beltsville (BE), VA (VA), NC (NC) locations. Mean bars within each year with no letter or the same letter are not significantly different ($P < 0.05$).

Table 1. Spatial measurements (mean \pm SEM) of *H. zea* feeding patterns on developing kernels in MON810 Bt and non-Bt corn ears

Measurements ^a	Bt	Non-Bt	t
Second instar			
Area (cm ²)	0.31 \pm 0.04	0.34 \pm 0.07	-0.33
Perimeter (cm)	2.9 \pm 0.2	2.97 \pm 0.44	-0.25
Compactness	28.4 \pm 1.9	27.7 \pm 2.4	0.17
Shape	0.54 \pm 0.03	0.50 \pm 0.05	0.64
Major axis (cm)	0.9 \pm 0.1	1.0 \pm 0.1	-0.47
Minor axis (cm)	0.5 \pm 0.0	0.5 \pm 0.1	0.46
Third instar			
Area (cm ²)	1.2 \pm 0.1	1.5 \pm 0.0	-1.49
Perimeter (cm)	7.0 \pm 0.5	6.6 \pm 0.7	0.45
Compactness	45.9 \pm 3.5	34.5 \pm 2.8	2.16
Shape	0.36 \pm 0.02	0.44 \pm 0.03	-2.00
Major axis (cm)	1.6 \pm 0.1	1.7 \pm 0.2	-0.79
Minor axis (cm)	1.0 \pm 0.1	1.1 \pm 0.1	-0.78
Fourth instar			
Area (cm ²)	2.7 \pm 0.2	2.3 \pm 0.2	1.48
Perimeter (cm)	11.0 \pm 0.8	8.5 \pm 0.4	2.92
Compactness	50.3 \pm 5.1	35.5 \pm 2.1	3.10
Shape	0.33 \pm 0.03	0.42 \pm 0.02	-2.70
Major axis (cm)	2.2 \pm 0.2	2.3 \pm 0.1	-0.46
Minor axis (cm)	1.4 \pm 0.1	1.3 \pm 0.1	0.96
Fifth instar			
Area (cm ²)	5.4 \pm 0.5	3.9 \pm 0.2	2.82
Perimeter (cm)	16.7 \pm 1.3	11.6 \pm 0.6	3.99
Compactness	51.8 \pm 5.3	36.9 \pm 2.6	2.62
Shape	0.32 \pm 0.06	0.40 \pm 0.02	-1.85
Major axis (cm)	4.4 \pm 1.4	2.8 \pm 0.1	1.17
Minor axis (cm)	2.0 \pm 0.2	1.8 \pm 0.1	1.20
Sixth instar			
Area (cm ²)	7.4 \pm 0.7	7.7 \pm 0.2	-0.42
Perimeter (cm)	19.2 \pm 1.3	17.0 \pm 0.5	1.32
Compactness	56.3 \pm 9.4	40.7 \pm 2.2	2.07
Shape	0.29 \pm 0.04	0.38 \pm 0.01	-2.16
Major axis (cm)	3.6 \pm 0.3	3.7 \pm 0.1	-0.42
Minor axis (cm)	2.7 \pm 0.2	2.7 \pm 0.1	0.046

The null hypothesis that the measurements within a row are the same is rejected at $P \leq 0.05$ (Student's *t*-test). *df* = 68, 95, 97, 85, 136 for 1st through 6th instars, respectively.

^a Area and perimeter measurements are totals of the surface area and perimeter of all feeding patches; compactness is a numerical expression that tends towards infinity as the pattern becomes less compact; shape is a calculation of how circular the feeding pattern is, ranges from 0 to 1 (perfect circle); major and minor axes are the longest and shortest distances across the feeding pattern.

for a longer period of time. The average instar pooled over sample dates in 1996 was 2.36 ± 0.13 for Bt corn and 4.19 ± 0.42 for non-Bt corn ($F = 14.5$; *df* = 1,24; $P = 0.001$). In 1997, effects of Bt corn on larval infestations were indicated by significant two-way interactions. For percentage of ears infested with larvae, the time \times location ($F = 3.2$; *df* = 4,196; $P = 0.015$) (Fig. 6a) and time \times corn type ($F = 11.9$; *df* = 4,196; $P = 0.001$) (Fig. 6b) means showed differences similar to those in 1996. Infestations in non-Bt ears were significantly higher during the first three sampling weeks but thereafter significantly lower than in Bt ears. Overall numbers of larvae per ear were much reduced in 1997; however, the same trends were observed as evident by significant interactions between time \times location ($F = 3.3$; *df* = 4,196; $P = 0.012$) (Fig. 7a) and time \times corn type ($F = 7.8$; *df* = 4,196; $P = 0.001$) (Fig.

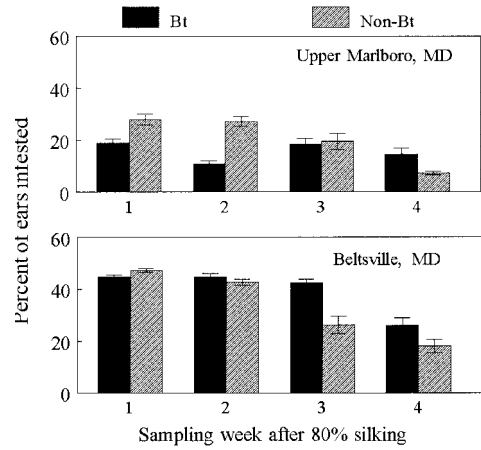


Fig. 4. Mean percentage (\pm SEM) of MON810 Bt and non-Bt ears infested with *H. zea* larvae at the Maryland locations in 1996. Data are given for four weekly sampling times after 80% silking.

7b). The progression of stadal development increased over time but was consistently delayed in Bt ears as indicated by the significant time \times corn type interaction for data ($F = 3.9$; *df* = 4,157; $P = 0.005$) (Fig. 8).

Larval Recruitment. Estimates of the total number of *H. zea* larvae developing through the early (first-third) and late larval (fourth-sixth) stages are given in Fig. 9. Bioassay results showed that larval development through all instars requires $\approx 50\%$ more time on diet incorporated with 20 g/liter of Bt leaf powder than larvae on non-Bt diet. Thus, cumulative degree-day requirements for *H. zea* development were adjusted by 150%. Values of 123 and 180 DD for early and late instars, respectively, were used to derive 'area under the curve' estimations of the number of larvae recruited in Bt corn.

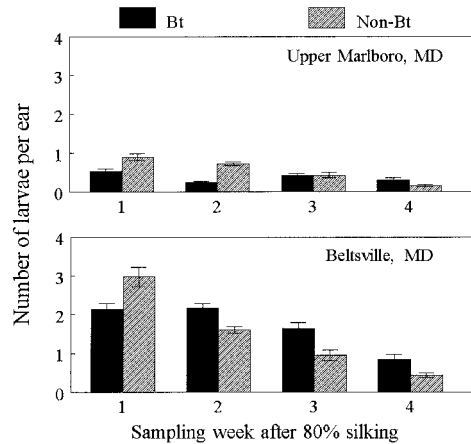


Fig. 5. Mean number (\pm SEM) of *H. zea* larvae per MON810 Bt and non-Bt ear at the Maryland locations in 1996. Data are given for four weekly sampling times after 80% silking.

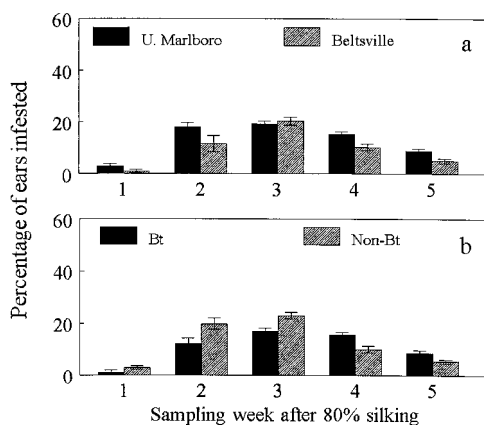


Fig. 6. Mean percentage (\pm SEM) of ears infested with *H. zea* larvae at (a) two Maryland locations and (b) in MON810 Bt and non-Bt plots over five sampling times in 1997.

The interaction effect of corn type by location on larval development was significant in both years. In 1996, differences in the number of larvae that passed through the early instars ranged from 5% more to 49% less in Bt compared with non-Bt corn depending on the location ($F = 4.12$; $df = 3,21$; $P = 0.0191$) (Fig. 9). For late instars, the number of larvae was reduced by 63–85% across the four locations ($F = 11.8$; $df = 3,18$; $P = 0.001$). For data pooled over locations, 24 and 70% fewer early and late larvae were recruited in Bt corn than in non-Bt corn, respectively (early larvae: $F = 16.9$; $df = 1,21$; $P = 0.001$; late larvae: $F = 191.3$; $df = 1,18$; $P = 0.001$). The change in the number of larvae passing through both age groups is an estimation of the real mortality from early to late instars. Overall mortality rates averaged 64 and 87% for larvae developing on non-Bt and Bt corn, respectively ($F = 34.4$; $df = 1,18$; $P = 0.001$).

In 1997, only the main effects of corn type and location on larval recruitment were significant. The number of early and late instars recruited in Bt corn

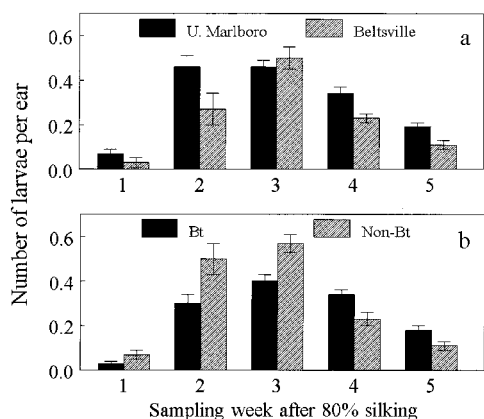


Fig. 7. Mean number (\pm SEM) of *H. zea* larvae per ear at (a) two Maryland locations and (b) in MON810 Bt and non-Bt plots over five sampling times in 1997.

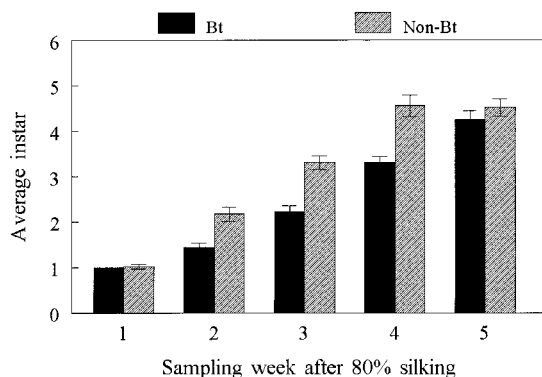


Fig. 8. Average instar stage (\pm SEM) of *H. zea* larvae in MON810 Bt and non-Bt ears over five sampling times in 1997. Data are pooled over both Maryland locations.

was reduced by 28% to 80% ($F = 45.64$; $df = 1, 15$; $P = 0.0001$) and 59% to 98% ($F = 49.43$; $df = 1, 15$; $P = 0.0001$), respectively (Fig. 9). Mortality from early to late larvae was consistent with results in 1997 and averaged 67 and 86% in Bt and non-Bt corn, respectively ($F = 26.9$; $df = 1, 15$; $P = 0.001$). Comparing results across locations, mortality rates ranged from 54 to 81% and 75–98% in non-Bt and Bt corn, respectively ($F = 15.1$; $df = 2,15$; $P = 0.001$).

Sublethal Effects. Laboratory studies showed that Bt corn significantly affected several fitness parameters of *H. zea* prepupae, pupae, and adults (Table 2). The development time to pupation of late sixth instars collected from Bt corn was delayed by 23%. However, the period of pupation and life span of adults were not affected. Average pupal weight of larvae that survived Bt corn was significantly reduced by 6%. Moths reared from larvae that survived Bt corn produced 35% fewer eggs per day than moths reared from non-Bt corn. The time effect was significant for daily egg production in both groups ($F = 5.5$; $df = 26, 51$; $P = 0.001$). Peak egg production occurred around 4–6 d and then declined to complete cessation after 26 d (Fig. 10). Although there was no significant time by corn type interaction, egg production from Bt-reared moths peaked a few days and declined at a faster rate than production from non-Bt moths. Fitness parameters that were not affected by previous feeding on Bt corn included mortality of sixth instars to the adult stage, sex ratio, and egg viability. In addition, there was no indication of any fitness disadvantage in F_1 larvae feeding on soybean. The survival and weight gain of F_1 larvae from Bt and non-Bt reared moths were statistically similar.

Discussion

As an ear-invading insect, *H. zea* is not a major yield-reducing pest of field corn and rarely the target of insecticide applications. However, any reductions in the incidence and intensity of kernel injury will have a direct beneficial effect on grain yield. More importantly, ear damage caused by *H. zea* and other insects has been linked to increased *Aspergillus* and

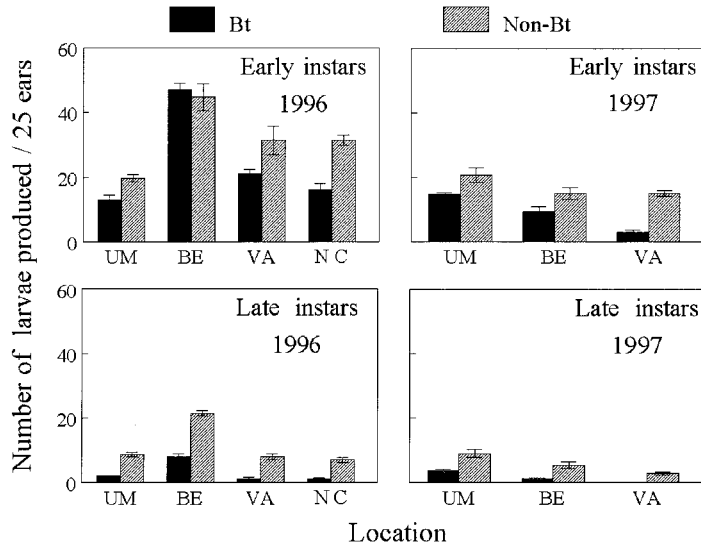


Fig. 9. Estimated number (\pm SEM) of *H. zea* larvae recruited through the early and late instar stages in MON810 Bt and non-Bt ears at Upper Marlboro (UM), Beltsville (BE), VA (VA), NC (NC) locations in 1996 and 1997.

Fusarium ear rots in field corn (Dowd 1998, Munkvold and McLean 1994, Smith and Riley 1992, Widstrom et al. 1975). Thus, reductions in kernel injury and the associated larval movement within ears may lower the risk of mycotoxin contamination (Dowd 2001, Dowd et al. 2000, Munkvold et al. 1999). MON810 Bt corn resulted in overall reductions in the percentage of damaged ears by 33% and in the amount of kernels consumed by 60%. However, the Bt-induced effects on *H. zea* and its damage varied significantly among study locations, partly because of differences in the overall level and timing of *H. zea* infestations. The ability of young larvae to successfully invade Bt ears may be density-dependent based on the number of eggs laid. Corn fields with higher moth activity should receive more eggs per silking ear, thus, the effects of Bt expression on density-dependent factors such as

cannibalism may play a disproportionate role in the survival of multiple ear infestations. Furthermore, exposure to the expressed toxin may be related to the condition of silk tissue at the time of egg hatch. Larvae hatching from eggs laid on wilted or brown silks may have a greater chance of surviving intoxication episodes because of reduced toxin expression in the senescent tissue. Plant stress may also contribute to the varied responses among locations. The NC site, which showed the lowest Bt-induced effects, was not irrigated, and thus rapid dry-down of silk tissue and possible acceleration of kernel maturation may have lowered toxin expression. All of these factors together can influence the efficacy of MON810 Bt corn in reducing *H. zea* infestations and ear damage.

The pattern of kernel damage caused by intoxicated *H. zea* in MON810 Bt corn was characteristically different. Larvae produced scattered, discontinuous patches of partially consumed kernels, which were arranged more linearly than the compact feeding patterns in non-Bt ears. The discontinuous patterns suggest that larvae in Bt ears are moving about sampling kernels more frequently than larvae in non-Bt ears. Dulmage et al. (1976) continuously exposed tobacco budworms to Bt endotoxins and found that they feed, become intoxicated and stop feeding, recover, and then feed again. Larvae of *Heliothis virescens* (F.), a species closely related to *H. zea*, have been shown to detect and avoid high levels of Bt toxin in diet (Gould et al. 1991). Generally, behavioral responses to Bt have been found to occur only as a response to toxic effects after ingestion and can contribute to the development of physiological tolerance to the toxin (Hoy et al. 1998). Thus, transgenic Bt crops should provide uniform distribution of a high dose toxin to maximize mortality of the target insect and allow less opportunity for movement from toxic to nontoxic parts of the

Table 2. Developmental and reproductive fitness parameters of prepupae, pupae, and adults reared from *H. zea* larvae surviving non-Bt and MON810 Bt field corn. Only data from the Maryland locations are pooled over 1996 and 1997

Fitness parameter	Bt	Non-Bt	df	P-value
Pupal wt (g)	0.432	0.458	1,711	<0.01
Developmental days (late 6 th instar to pupation)	7.2	6.1	1,715	<0.01
Developmental days (pupae to adult emergence)	11.3	11.3	1,577	0.33
Percent mortality (late 6 th instar to adult emergence)	13.7	20.9	1	0.34
Sex ratio (M:F)	1.24:1	1.09:1	1	0.43
Days of adult life	13.4	12.9	1,338	0.56
Fecundity (eggs/female/day)	58	89	1,632	0.02
Percent eclosion	49.5	46.6	1,33	0.34
Weight of F ₁ larvae on soybean (g)	0.346	0.354	1,4	0.85

The null hypothesis that the fitness parameter within a row are the same is rejected at $P < 0.05$ based on ANOVA or chi-square.

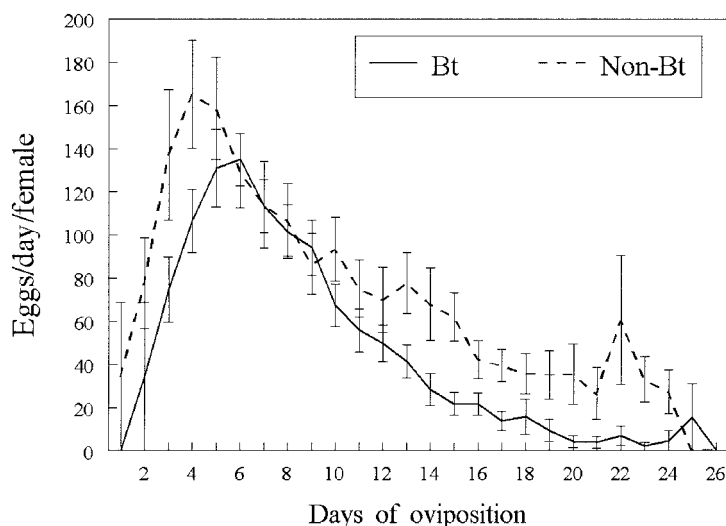


Fig. 10. Average daily fecundity over the reproductive life span of *H. zea* female adults reared from surviving larvae collected from MON810 Bt and non-Bt ears. Data are pooled over both Maryland locations in 1997.

plant (Hoy et al. 1998). This condition is not true in Bt corn ears because not all kernels express the same level of endotoxin. Allele segregation results in a variable number of Bt genes among kernels, resulting in $\approx 25\%$ of kernels not expressing at all. Thus, the spatial heterogeneity of toxin distribution among kernels may provide an opportunity for development of behavioral responses in *H. zea* to avoid toxin.

MON810 corn suppressed the development and recruitment of *H. zea* to late instar stages by at least 75%. These results are in agreement with the study by Storer et al. (2001) that showed reductions of 60–85% in larval survival to the prepupal stage. This level of control is considered indicative of a moderate dose-response compared with the high dose expression exerted on *O. nubilalis*. The difference is partly because of the lower expression of the endotoxin in the silk tissue and kernels, where *H. zea* feeds, compared with levels in the leaves and stalk where *O. nubilalis* primarily feeds. The range of Cry1Ab toxin level in MON810 corn is only 0.4–0.7 g/g in the kernels compared with 5.2–10.6 g/g in leaf tissue (U.S. Environmental Protection Agency 1997). In addition, *H. zea* is less susceptible to Cry1Ab endotoxin than *O. nubilalis* (MacIntosh et al. 1990), and apparently many larvae can recover from intoxication episodes and eventually develop to more tolerant instars. Thus, as pointed out in Storer et al. (2001), moderate dose expression may increase the risk of resistance development by *H. zea* in areas where MON810 Bt corn is widely adopted and where *H. zea* overwinters successfully to pass resistant alleles to the next generation. The lack of a high dose could allow heterozygous insects with one copy of the resistance allele to survive, thus increasing the frequency of resistance genes in the population. However, Gould and Tabashnik (1998) suggest that moderate Bt expression combined with large effective refuge size may slow evolution of

resistance. The current insect resistance management plan for Bt field corn requires growers to plant a 20% non-Bt refuge outside cotton-growing areas or a 50% non-Bt refuge in cotton-growing areas (National Corn Growers Association 2001). Although designed primarily to reduce the risks of *O. nubilalis* resistance, this plan is expected to maintain susceptibility of *H. zea*, particularly in areas of high overwintering mortality, few generations per year, and low or no Bt cotton use. Furthermore, the presence of wild plants and other crop hosts in many corn production areas may serve to increase the effective refuge size for *H. zea*, and thus, compensate for the moderate expression.

The sublethal effects of moderate toxin expression in MON810 Bt corn resulted in several fitness disadvantages of *H. zea*. Specifically, larval development to late sixth instars required 50% more time on diet incorporated with MON810 leaf powder than it did for larvae on non-Bt diet. Larvae found in Bt ears were usually two or more stadia behind the development of larvae in non-Bt ears. Of the late sixth instars collected from Bt ears, development to pupation was delayed by 23% and pupal weight was reduced by 6%. Because most larvae had completed their feeding and were ready to pupate when collected from ears, the delay in development to pupation carried over from the cumulative effects of intoxication episodes of larvae feeding in Bt ears. These responses corroborate the results of Storer et al. (2001), except in this study the pupal weights of Bt larvae were reduced by 33%. Sims et al. (1996) described sublethal effects of Cry1Ab protein on *H. zea* as similar to the effects of larvae feeding on corn plants with native genes expressing antibiosis traits. Many examples of antibiosis-type effects have been reported for *H. zea* feeding on classically bred resistant corn plants (Bennett et al. 1967, Chambliss and Wann 1971, Wiseman and Widstrom 1992a, b, Wiseman and Isenhour 1994). Such effects

include reduced larval and pupal weight, increased development time, and delayed pupation. Although certain fitness parameters in this study were measured under laboratory conditions, results provide insights into how well *H. zea* survivors of Bt corn will perform on nonexpressing hosts and successfully contribute genes to the next generation. It is clear that *H. zea* survivors may not develop normally for a period of time after feeding on Bt corn. Any delay in prepupal development could have a major impact on survival if it takes longer for larvae to enter the soil and form pupal cells. An extended prepupal period could increase exposure to natural enemies and abiotic mortality factors. Also, the combined effects of developmental delays may result in temporal asynchrony of *H. zea* moths produced from Bt and non-Bt corn. Current resistance management strategies using refuges are based on the assumption that susceptible individuals from the refuge mate randomly with the selected population (Caprio 1998). Delays in *H. zea* development because of Bt corn feeding and asynchrony of adult emergence could result in susceptible individuals mating before resistant adults emerge (Gould 1998). Although this is a possible scenario, it is unknown whether resistant or partially resistant individuals will actually exhibit delayed development.

The most significant effect of sublethal exposure to MON810 Bt was shown in the fecundity of *H. zea*. Average daily oviposition by moths reared from late sixth instars collected from Bt corn was 35% less than the reproductive output of moths from non-Bt corn. The reduced oviposition occurred primarily through the first 6 d after emergence and may have been related to decreased pupal size. Fecundity has been linked to body size in a related species, *Helicoverpa armigera* Hübner (Reed 1965). Although reproductive output was reduced, there were no apparent carryover effects on egg hatch or F_1 larval performance on soybean. Reduced recruitment, delays in development, and reduced fecundity of *H. zea* may also have an influence on the population size of subsequent generations in other host crops. In many areas, corn is a major source of *H. zea* moths that move to alternate hosts, such as cotton and soybean, later in the season as corn matures. Fitt (1989) and Kennedy et al. (1987), using computer simulations, reported that infestation rates in other host crops could be reduced if antibiosis traits in corn suppress *H. zea* populations.

In summary, this study has shown that *H. zea* exhibited both lethal and sublethal effects as a result of feeding on MON810 Bt corn. These effects translate into real benefits of reduced kernel damage, lowered risks of mycotoxins, and potential localized suppression of *H. zea* populations. However, further studies are needed to ascertain whether resistant individuals exhibit the same fitness disadvantages and if Bt-induced effects will significantly accelerate the evolution of resistance. In addition, many contributing factors, including initial gene frequency, functional dominance, and number of resistance genes, associated with the evolution of *H. zea* resistance to Cry1Ab toxin remain unknown at this time (ILSI 1999). This

study suggests that the moderate dose effects of MON810 Bt corn and the spatial heterogeneity of toxin distribution among kernels could increase the additive genetic variance for both physiological and behavioral resistance in *H. zea* populations. Given this and the fact that many factors are unknown, Gould (1998) has suggested that Bt crops expressing moderate dose control of target pests should be deployed in a very judicious manner.

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